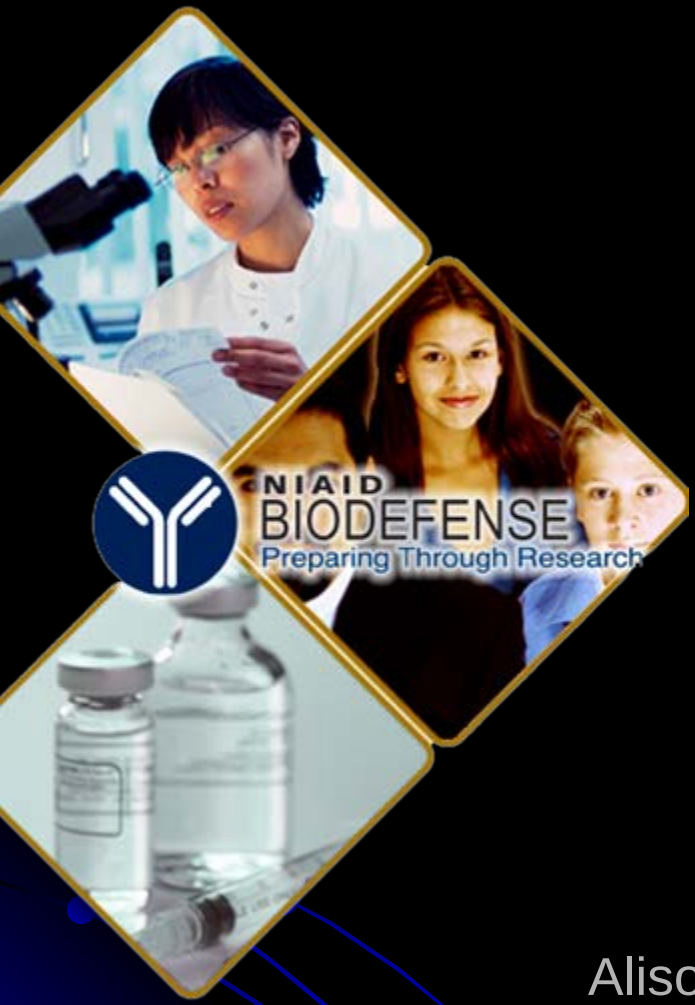

Large-Scale Antibody and T cell Epitope Discovery Contracts

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Large-Scale Antibody and T cell Epitope Discovery Contracts

- Main goal: identify immune epitopes from NIAID Category A, B, and C priority pathogens
- 14 contracts awarded 2004: 12 T cell, 1 antibody, 1 both
- Contractors required to submit their data to the Immune Epitope Database (IEDB)

Epitope Discovery Contracts - Summary

- ***Arenaviruses:*** La Jolla Institute for Allergy and Immunology
- ***Bacillus anthrasis:*** Imperial College, UK (also yersinia pestis)
- ***Botulism toxins:*** Scripps Research Institute
- ***Ebola:*** Duke (also vaccinia and multi-drug resistant TB)
- ***Francisella tulerensis:*** UNC Chapel Hill
- ***Influenza:*** Virginia Mason Research Ctr (also clostridium tetani, anthrax); Univ of OK Hlth Sci Ctr (also west nile virus, coxiella burnetti)
- ***Multi-drug resistant TB:*** Oregon Health and Sciences University
- ***Vaccinia/Variola:*** Vanderbilt University; Torrey Pines Institute for Molecular Studies; La Jolla Institute for Allergy and Immunology
- ***Yellow Fever:*** Johns Hopkins University (also hanta virus, hep. A, anthrax, rabies, arenaviruses, West Nile, SARS)
- ***all NIAID A-C pathogens:*** Ctr for Biological Sequence Analysis ; Denmark; University of Copenhagen

La Jolla Institute for Allergy and Immunology

- PI: Alessandro Sette
- Arenavirus MHC Class I and Class II epitopes in human, mouse, and non-human primates
- Arenavirus species: Lassa, LCM, Junin, Machupo, Guararito, Sabia, and Whitewater Arroyo
- Proteins: L, NP, GPC, and Z (L= RNA polymerase, NP=Nucleoprotein, GPC=glycoprotein, Z=zinc-binding protein)
- 8 human HLA supertypes: HLA A1, A2, A3, A24, B7, B44, DR, and DR3; 7 non-human : Mouse K^b, D^b , and IA^b; Mamu A*01, Mamu B*17, Mamu DR*w201, and Mamu DR*0406
- Validation: MHC-Peptide Binding assays; ELISPOT; Recombinant Vaccinia Constructs; cytotoxicity assays

Imperial College London

- PI – Daniel Altmann
- MHC class II T cell epitopes : protective antigen (PA) and lethal factor (LF) from *Bacillus anthracis,* and virulence factor (V) and capsular fraction one antigen (F1) from *Yersinia pestis*
- Mapping - HLA Class II transgenic mice (infection or recombinant proteins)
- Anthrax *In vivo* validation: PBMC from rPA vaccine, DNA vaccine (mouse), or natural infections (Turkey)
- *Yersinia pestis* : HLA tg mouse studies only
- Antibody epitopes: peptide arrays, serum from rPA -vacciniated military and patients recovered from cutaneous anthrax

Scripps Research Institute

- PI – Kim Janda
- Protective monoclonal antibody epitopes on Clostridium botulinum neurotoxins A, B, and E
- Method: human scFv-phage library to select neurotoxin-specific human neutralizing mAbs
- Antibodies used as probes to discover epitopes
- Antigenicity and immunogenicity: validated by *in vitro* and *in vivo* assays (animals)

Duke University Medical School

- PI – Kent Weinhold
- CD8+ T cell epitopes in Ebola glycoprotein, early and immediate early genes for poxviruses, and multi-drug resistant M. tuberculosis (Itopia/Beckman)
- Immunogenicity in vitro:
  - γ-IFN production and cytotoxicity
  - MHC Class I tetramer binding assay
- Immunogenicity in vivo (PBMC)
  - Ebola: samples collected in NIAID VRC-sponsored vaccine trial
  - Pox : Dryvax vaccinees
  - TB: patients recovering from active infection

University of North Carolina, Chapel Hill

- PI – Jeffery Frelinger
- Francisella tularensis MHC class I and II T cell epitopes
- T Cell Antigen Discovery (T-CAD) assay: genomic fragments of FT in E. coli expression vector. Purified proteins coupled to beads, fed to APC
- Screen CD4+ and CD8+ T cell hybridomas from mice immunized with killed or live Francisella (live vaccine strain, group B or group A)
- HLA Class I and Class II tg mice - define epitopes associated with human MHC; protection studies

Benaroya Research Institute, Virginia Mason Research Center

- PI – William Kwok
- Identification of CD4+ T cell epitopes for Clostridium tetani, influenza, and Bacillus anthracis antigens
- Method: multiplexing tetramer guided epitope mapping (TGEM) ; 28 different HLA class II alleles
- Antigens: Tetanus toxoid; hemaglutinin (HA), matrix (M), and nucleprotein (NP) influenza A; HA influenza B; protective antigen (PA) Bacillus anthracis
- Validation: peptide binding assay, cloning of T cell lines, proliferation assays and ELISPOT assays

University of Oklahoma Health Sciences Center

- PI – William Hildebrand
- HLA class I peptide epitopes: West Nile Virus, Influenza, and *Coxiella burnetti*
- Characterizing epitopes generated in infected cells (expressing either soluble HLA A*201 or B*0702). Eluted peptides will be mapped by HPLC/mass spectrometry to identify T cell epitopes present during infection.
- Validation: T cells from infected individuals

Oregon Health and Science University

- PI – David Lewinsohn
- MHC Class I restricted T cells in Mycobacterium tuberculosis (Mtb), also nonclassical MHC (Class Ib, CD1)
- Minimal epitope and HLA restriction determined; Peptide pools : ~ 400 genes of Mtb
- T cell clones to Mtb from active Mtb or latently infected Mtb donors tested with peptide pools, DCs expressing Mtb genes, or Mtb cell wall antigens (ELISPOT)
- Clinical correlations: PBMC from active TB subjects and household contacts in Uganda; samples from CDC household contact study

La Jolla Institute for Allergy and Immunology

- PI – Alessandro Sette
- vaccinia virus MHC Class I and Class II epitopes in human, mouse, and non-human primates
- Overlapping peptides - all vaccinia-derived open reading frames
- MHC restriction: HLA DR supertype and 4 main HLA A supertypes (ELISPOT assays)
- Validate: high throughput MHC binding assays; in vitro T cell assays; and in vivo assays (Tg mice)
- Antigen subsets recognized by PBMC from vaccinees: more exhaustive search for HLA A1, A2, A3, A24 and DR supertype, and HLA B7, B44, and DR3 supertype epitopes
- Corresponding variola virus sequences tested in recall ELISPOT assays

Torrey Pines Institute for Molecular Studies

- PI - Clemencia Pinilla
- T cell epitopes recognized by CD4+ and CD8+ T cells from Vaccinia immunized donors
- Positional scanning-synthetic combinatorial libraries (PS-SCL) - series of sub-libraries in which each position in a peptide is defined
- Validation: Human T cell lines and clones (vaccinated with Dryvax or MVA); LCLs/overlapping peptides

Vanderbilt University

- PI – Sebastian Joyce
- HLA class I peptide epitopes from Vaccinia
- Characterizing epitopes generated in infected cells (expressing either soluble HLA A*201 or B*0702). Eluted peptides will be mapped by HPLC/mass spectrometry to identify T cell epitopes present during infection.
- Validation: tetramer staining of T cells from vaccinated and naive individuals

Johns Hopkins University Medical School

- PI – Tom August
- Computational modeling (Hidden Markov, Artificial Neural Networks) to define MHC Class I and class II-restricted T cell epitopes for numerous Category A-C pathogens: Yellow Fever Hepatitis A, West Nile, rabies, Arenaviruses, Anthrax, Hanta
- Collection and storage of PBMC samples from clinical cohort (yellow fever, hep. A, rabies; Brazil); WNV – mouse T cells
- Test ex vivo epitope-specific T cell responses from clinical cohort samples, compare/validate predicted epitopes (ELISPOT)
- Test multi-epitope vaccines (MHC class I and II) in HLA transgenic mice

Technical University of Denmark

- PI –Ole Lund
- MHC class I epitope binding algorithms –based on antigen processing/presentation (ANN – MHC binding; proteosomal cleavage site; peptide/TAP binding; trimming by ER aminopeptidases)
- Pathogens: influenza; vaccinia; Bacillus anthracis; Clostridium botulinum; Yersinia pestis; Francisella tularensis; Hantaan virus; Rift Valley Fever; Dengue; Ebola; Marburg; Multi-drug resistant TB; and three Arenaviruses
- Methods: epitope prediction from genomic sequences
- Validation: peptide/ HLA-class I binding assays; T cell recognition from healthy donors (influenza, vaccinia)

University of Copenhagen

- PI – Soren Buus
- Integrated suite of MHC class I epitope predictive tools, based on antigen processing and presentation events
- Study all NIAID category A-C pathogens where primary sequences available in public databases
- Artificial neural networks - primary tools; hidden Markov models may also be used
  - Use existing databases to generate preliminary predictions
  - Use predictions to select a set of data points that will complement existing data
  - Generate new information-rich quantitative data with prediction tools
- Validation: homology modeling (HLA/peptide); ELISA-driven binding assay (recombinant HLA)